(12) UK Patent Application (19) GB (11) 2 388 540 (13) A

(43) Date of A Publication 19.11.2003

(21) Application No: 0211396.7

(22) Date of Filing: 17.05.2002

(71) Applicant(s):
Bayer AG
(Incorporated in the Federal Republic of Germany)
D-51368 Leverkusen,
Federal Republic of Germany

(72) Inventor(s):
Kevin Bacon
Yoshihisa Manabe
Akihiko Watanabe
Takashi Iino
Hiromi Sugimoto

(74) Agent and/or Address for Service:
Carpmaels & Ransford
43 Bloomsbury Square, LONDON,
WC1A 2RA, United Kingdom

(51) INT CL[7]:
A61K 31/403 , A61P 1/08 11/06 35/02 37/08

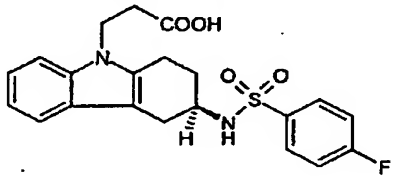
(52) UK CL (Edition V):
A5B BHA B180 B46Y B461 B48Y B480 B51X B55Y B551 B57Y B575 B58Y B586 B61Y B616 B65X B65Y
U1S S1310 S1313

(56) Documents Cited:
Chemical Abstracts Acc. No. 2000:353360.
Chemical Abstracts Acc. No. 2002:34819.

(58) Field of Search:
UK CL (Edition V) **A5B**
INT CL[7] **A61K, A61P**
Other: **Online: CAS-ONLINE; EPODOC; WPI; PAJ**

(54) Abstract Title: **New use of Ramatroban**

(57) The invention provides the compound represented by the formula (I):



or acceptable salt thereof as a CRTH2 modulator.
The compounds of the formula (I): (+)-(3R)-3-(4-fluorobenzenesulfonamido)-1,2,3,4-tetrahydrocarbazole-9-propionic acid or a pharmaceutically acceptable salt thereof has excellent CRTH2 antagonistic activity and is useful for the prophylaxis and treatment of diseases associated with the activity, in particular for the treatment of allergic diseases, such as asthma, allergic rhinitis, allergic conjuvatitis. In addition, eosinophil-related diseases, such as Churg-Strauss syndrome and sinusitis should be included as possible targets of a CRTH2 modulator. Moreover, basophil-related diseases, such as basophilic leukemia and basophilic leukocytosis should be also included.

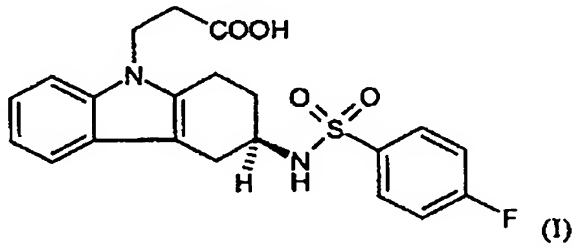
GB 2 388 540 A

New use of Ramatroban

DETAILED DESCRIPTION OF INVENTION

TECHNICAL FIELD

The present invention relates to a CRTH2 modulator. More specifically, the present invention relates to a CRTH2 modulator, which comprises the compound represented by the formula (I):



(I)

(+)-(3R)-3-(4-fluorobenzenesulfonamido)-1,2,3,4-tetrahydrocarbazole-9-propionic acid (Bay u 3405, ramatroban) or a pharmaceutically acceptable salt thereof, as an active ingredient. The compound is useful in therapy in particular in the treatment or prevention of allergic diseases, such as asthma, allergic rhinitis and allergic conjunctivitis; eosinophil-related diseases, such as Churg-Strauss syndrome and sinusitis; and basophil-related diseases, such as basophilic leukemia and basophilic leukocytosis in human and other mammals.

BACKGROUND ART

Ramatroban is known as having platelet aggregation inhibitory activity and thromboxane A_2 receptor antagonistic activity and useful in the treatment or prevention of thrombosis, thromboembolism and ischaemia. It is also known to be useful as an antiasthma agent, antiallergic agent and useful in allergic dermatitis (Japanese patent Tokkyohei 4-50301, Japanese laid-open patent publication

Tokkaihei8-175991). However, it is not known that ramatroban has CRTH2 modulatory (antagonistic or agonistic) activity.

CRTH2 is a G-protein-coupled chemoattractant receptor, expressed on Th2 cells(Nagata et al. J. Immunol., **162**, 1278-1286, 1999), eosinophils and basophils (Hirai et al., J. Exp. Med., **193**, 255-261, 2001).

Th2-polarization has been seen in allergic diseases, such as asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis (Romagnani S. Immunology Today, **18**, 263-266, 1997; Hammad H. et al., Blood, **98**, 1135-1141, 2001). Th2 cells regulate allergic diseases by producing Th2 cytokines, such as IL-4, IL-5 and IL-13 (Oriss et al., J. Immunol., **162**, 1999-2007, 1999; Viola et al., Blood, **91**, 2223-2230, 1998; Webb et al., J. Immunol., **165**, 108-113, 2000; Dumont F.J., Exp. Opin. Ther. Pat., **12**, 341-367, 2002). These Th2 cytokines directly or indirectly induce migration, activation, priming and prolonged survival of effector cells, such as eosinophils and basophils, in allergic diseases (Sanz et al., J. Immunol., **160**, 5637-5645, 1998; Pope et al., J. Allergy Clin. Immunol., **108**, 594-601, 2001; Teran L.M., Clin. Exp. Allergy, **29**, 287-290, 1999).

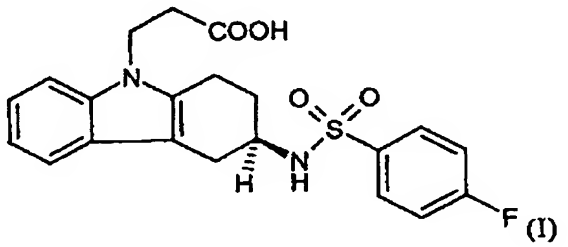
PGD_2, a ligand for CRTH2, is produced from mast cells, another important effector cells in allergic diseases (Nagata et al., FEBS Lett. **459**, 195-199, 1999; Hirai et al., J. Exp. Med., **193**, 255-261, 2001). PGD_2 induces migration and activation of Th2 cells, eosinophils, and basophils, via CRTH2 (Hirai et al., J. Exp. Med., **193**, 255-261, 2001; Gervais et al., J. Allergy Clin. Immunol., **108**, 982-988, 2001).

Therefore, antagonists which inhibit the binding of CRTH2 and PGD_2 should be useful for the treatment of allergic diseases, such as asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis. In addition, several experimental evidence has demonstrated the contribution of eosinophils in sinusitis (Hamilos et al., Am. J. Respir. Cell and Mol. Biol., **15**, 443-450, 1996; Fan et al., J. Allergy Clin. Immunol., **106**, 551-558, 2000), and Churg-Strauss syndrome (Coffin et al., J. Allergy Clin.

Immunol., **101**, 116-123, 1998; Kurosawa et al., Allergy, **55**, 785-787, 2000). In the tissues of these patients, mast cells can be observed to be colocalized with eosinophils (Khan et al., J. Allergy Clin. Immunol., **106**, 1096-1101, 2000). It is suggested that PGD_2 production from mast cells induces the recruitment of eosinophils. Therefore, CRTH2 modulators are useful not only for the treatment of allergic diseases, but also for the treatment of other eosinophil-related diseases. CRTH2 also may be involved in the recruitment of basophils in some basophil-related diseases, because of high expression of CRTH2 on basophils.

SUMMARY OF THE INVENTION

This invention is to provide a CRTH2 modulator, which comprises the compound represented by the formula (I):



(I)

(+)-(3R)-3-(4-fluorobenzenesulfonamido)-1,2,3,4-tetrahydrocarbazole-9-propionic acid or a pharmaceutical acceptable salt thereof as an effective agent.

The compound represented by the formula (I) shows excellent CRTH2 antagonistic activity. They are, therefore, suitable especially for the prophylaxis and treatment of diseases associated with CRTH2 activity, in particular for the treatment or prevention of allergic diseases, such as asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis; eosinophil-related diseases, such as Churg-Strauss syndrome and sinusitis; and basophil-related diseases, such as basophilic leukemia and basophilic leukocytosis.

EMBODIMENT OF THE INVENTION

It is a discovery of the present invention that ramatroban or a pharmaceutically acceptable salt thereof has CRTH2 antagonistic activity and can be regulated to control diseases that are caused by aberrant activity of this receptor and diseases whose symptoms can be ameliorated by stimulating or inhibiting the activity of CRTH2.

More specifically, ramatroban and the salt thereof inhibit the binding of $[^3H]PGD_2$ to CRTH2 transfectants in a dose-dependent manner. Ramatroban and its salts inhibit PGD_2-induced Ca^{2+} flux of $CRTH_2$ transfectants in a dose-dependent manner. Ramatroban and its salts inhibit PGD_2-induced chemotaxis of eosinophils and $CD4^+$ T cells in a dose-dependent manner. These results demonstrate that ramatroban and its analogues can inhibit migration of CRTH2-expressing cells, such as eosinophils, basophils and Th2 cells, by the inhibition of the binding of $[^3H]PGD_2$ to CRTH2.

Therefore, ramatroban and its analogues should be very useful for the treatment of allergic diseases, such as asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis, eosinophil-related diseases, such as Churg-Strauss syndrome and sinusitis, and basophil-related diseases, such as basophilic leukemia and basophilic leukocytosis.

Ramatroban has been well known and can be prepared by the methods described in Japanese patent Tokkyohei 4-50301.

Typical salts of the compound shown by the formula (I) include salts prepared by reaction of the compounds of the present invention with a mineral or organic acid, or an organic or inorganic base. Such salts are known as acid addition and base addition salts, respectively.

Acids to form acid addition salts include inorganic acids such as, without limitation, sulfuric acid, phosphoric acid, hydrochloric acid, hydrobromic acid, hydriodic acid and the like, and organic acids, such as, without limitation, p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like.

Base addition salts include those derived from inorganic bases, such as, without limitation, ammonium hydroxide, alkaline metal hydroxide, alkaline earth metal hydroxides, carbonates, bicarbonates, and the like, and organic bases, such as, without limitation, ethanolamine, triethylamine, tris(hydroxymethyl)aminomethane, and the like. Examples of inorganic bases include, sodium hydroxide, potassium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like.

The compound of the present invention or a salts thereof, depending on its substituents, may be modified to form lower alkylesters or known other esters; and/or hydrates or other solvates. Those esters, hydrates, and solvates are included in the scope of the present invention.

The compound of the present invention may be administered in oral forms, such as, without limitation normal and enteric coated tablets, capsules, pills, powders, granules, elixirs, tinctures, solution, suspensions, syrups, solid and liquid aerosols and emulsions. They may also be administered in parenteral forms, such as, without limitation, intravenous, intraperitoneal, subcutaneous, intramuscular, and the like forms, well-known to those of ordinary skill in the pharmaceutical arts. The compounds of the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using transdermal delivery systems well-known to those of ordinary skilled in the art.

The dosage regimen with the use of the compounds of the present invention is selected by one of ordinary skill in the arts, in view of a variety of factors, including,

without limitation, age, weight, sex, and medical condition of the recipient, the severity of the condition to be treated, the route of administration, the level of metabolic and excretory function of the recipient, the dosage form employed, the particular compound and salt thereof employed.

The compounds of the present invention are preferably formulated prior to administration together with one or more pharmaceutically-acceptable excipients. Excipients are inert substances such as, without limitation carriers, diluents, flavoring agents, sweeteners, lubricants, solubilizers, suspending agents, binders, tablet disintegrating agents and encapsulating material.

Yet another embodiment of the present invention is pharmaceutical formulation comprising a compound of the invention and one or more pharmaceutically-acceptable excipients that are compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. Pharmaceutical formulations of the invention are prepared by combining a therapeutically effective amount of the compounds of the invention together with one or more pharmaceutically-acceptable excipients therefore. In making the compositions of the present invention, the active ingredient may be mixed with a diluent, or enclosed within a carrier, which may be in the form of a capsule, sachet, paper, or other container. The carrier may serve as a diluent, which may be solid, semi-solid, or liquid material which acts as a vehicle, or can be in the form of tablets, pills, powders, lozenges, elixirs, suspensions, emulsions, solutions, syrups, aerosols, ointments, containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

For oral administration, the active ingredient may be combined with an oral, and non-toxic, pharmaceutically-acceptable carrier, such as, without limitation, lactose, starch, sucrose, glucose, sodium carbonate, mannitol, sorbitol, calcium carbonate, calcium phosphate, calcium sulfate, methyl cellulose, and the like; together with, optionally, disintegrating agents, such as, without limitation, maize, starch, methyl

cellulose, agar bentonite, xanthan gum, alginic acid, and the like; and optionally, binding agents, for example, without limitation, gelatin, natural sugars, beta-lactose, corn sweeteners, natural and synthetic gums, acacia, tragacanth, sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like; and, optionally, lubricating agents, for example, without limitation, magnesium stearate, sodium stearate, stearic acid, sodium oleate, sodium benzoate, sodium acetate, sodium chloride, talc, and the like.

In powder forms, the carrier may be a finely divided solid which is in admixture with the finely divided active ingredient. The active ingredient may be mixed with a carrier having binding properties in suitable proportions and compacted in the shape and size desired to produce tablets. The powders and tablets preferably contain from about 1 to about 99 weight percent of the active ingredient which is the novel composition of the present invention. Suitable solid carriers are magnesium carboxymethyl cellulose, low melting waxes, and cocoa butter.

Sterile liquid formulations include suspensions, emulsions, syrups and elixirs. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable carrier, such as sterile water, sterile organic solvent, or a mixture of both sterile water and sterile organic solvent.

The active ingredient can also be dissolved in a suitable organic solvent, for example, aqueous propylene glycol. Other compositions can be made by dispersing the finely divided active ingredient in aqueous starch or sodium carboxymethyl cellulose solution or in a suitable oil.

The formulation may be in unit dosage form, which is a physically discrete unit containing a unit dose, suitable for administration in human or other mammals. A unit dosage form can be a capsule or tablets, or a number of capsules or tablets. A "unit dose" is a predetermined quantity of the active compound of the present invention, calculated to produce the desired therapeutic effect, in association with

one or more excipients. The quantity of active ingredient in a unit dose may be varied or adjusted from about 0.1 to about 1000 milligrams or more according to the particular treatment involved.

Typical oral dosages of the compound of the present invention, when used for the indicated effects, will range from about 1 mg /kg/day to about 10 mg/kg/day. The compounds of the present invention may be administered in a single daily dose, or the total daily dose may be administered in divided doses, two, three, or more times per day. Where delivery is via transdermal forms, of course, administration is continuous.

EXAMPLES

The present invention will be described as a form of examples. In the example below, all quantitative data, if not stated otherwise, relate to percentages by weight.

EXAMPLE 1

Human CRTH2 was amplified from human eosinophil cDNA using the primers described in Nagata et al. J. Immunol., 162, 1278-1286, 1999 and cloned into pEAK vector (Edge Bio Systems). The cloned CRTH2 gene (40 μg), inserted into pEAK vector, was transfected into host cells, such as L1.2 cells, K562 cells, and HEK293 cells, at a cell density of $1x10^7$ cells/50 μl, by using electroporation apparatus (Gene Pulser II, BioRad) at 250V/1,000 μF. One day after the transfection, puromycin (1 μg/ml, Sigma) was added into the cell culture plates. Two weeks after the transfection, grown cells were picked up for further growth.

EXAMPLE 2

CRTH2 transfectants were washed once with PBS and resuspended in binding buffer (25 mM HEPES pH 7.6, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA, 0.1% NaN_3). 100 μl

of cell suspension (2 x 10^5 cells), [^{3}H]-labeled PGD_2, and various concentrations of the ramatroban were then mixed in a 96-well U-bottom polypropylene plate and incubated for 60 min at room temperature to allow binding to occur. After incubation, the cell suspension was transferred to a filtration plate (#MAFB, Millipore) and washed 3 times with binding buffer containing 0.5 M NaCl. Scintillant was added to the filtration plate, and radioactivity remaining on the filter was measured by TopCount (Packard), a scintillation counter. Non-specific binding was determined by incubating the cell suspension and [^{3}H]-labeled PGD_2 in the presence of 500 nM of unlabeled PGD_2. Puromycin-resistant L1.2 transfectants bound to [^{3}H]-labeled PGD_2 with high affinity (K_D = 6.3 nM). Ramatroban inhibited the binding of [^{3}H]-labeled PGD_2 to CRTH2 transfectants in a dose-dependent manner, with IC_{50} = 45 nM.

EXAMPLE 3

Ca^{2+} loading buffer was prepared by mixing 5 µl of Fluo-3AM (2 mM in DMSO, final 1 µM, Molecular Probes) and 10 µl of pluronic F-127 (Molecular Probes) and diluting the resulting mixture in 10 ml of Ca^{2+} assay buffer (20 mM HEPES pH 7.6, 0.1% BSA, 1 mM probenecid, Hanks' solution). The CRTH2 transfectanteds cells which were prepared in Example 1 were washed with PBS, resuspended in Ca^{2+} loading buffer at 1 x 10^7 cells/ml, and incubated for 60 min at room temperature. After incubation, cells were washed and resuspended in Ca^{2+} assay buffer, then dispensed into transparent-bottom 96-well plates (#3631, Costar) at 2 x 10^5 cells/well. Cells were incubated with various concentrations of ramatroban for 5 minutes at room temperature. The emitted 480 nm fluorescence was measured on FDSS6000, a Ca^{2+} -measurement apparatus (Hamamatsu Photonics, Hamamatsu, Japan). The transfectant showed PGD_2-induced Ca^{2+} flux in a concentration-dependent manner. Ramatroban inhibited 10 nM PGD_2-induced Ca^{2+} flux in a dose-dependent manner, with IC_{50} = 30 nM.

EXAMPLE 4

Animal model: Eosinophilia is induced in the airway of cynomolgus monkeys (*Macaca fascicularis*, weighing 4.0-9.0 kg) by PGD_2 inhalation (10-100 ng/ml, 10 min.) with the use of an appropriate nebulizer, such as an ultrasonic nebulizer (TUR-3200, Nihon Kohden). Ramatroban is given 1 hr before the inhalation. A pediatric fiberoptic bronchoscope (Olympus, model BF3C30) is guided past the carina into the distal lung until the tip of the bronchoscope is wedged in the bronchoalveolar region. A 15 ml aliquot of saline is instilled slowly down one channel of the bronchoscope followed by 2-3 ml of air to completely empty the bronchoscope channel. The fluid is aspirated back into a syringe. Typical recovery volumes are greater than 60%. The volume is recorded. Cell number is counted and cell differentiation is determined by using stained cytospin specimens.

EXAMPLE 5

Human polymorphonuclear cells were isolated from heparinized venous blood of healthy donors by laying the blood on Mono-Poly Resolving Medium (ICN Biomedicals, Co.Ltd) and centrifuging it at 400 x g for 30 min. at room temperature. After centrifugation, eosinophils were purified from the the lower layer of polymorphonuclear cells by CD16-negative selection using anti-CD16-conjugated magnetic beads (Miltenyi Biotech GmbH).

Human eosinophils were washed with PBS and resuspended in chemotaxis buffer (20 mM HEPES pH 7.6, 0.1% BSA, Hanks' solution) at 6×10^6 cells/ml. Fifty µl of the cell suspension (3×10^5 cells/well) was then dispensed into the upper chamber and 30 µl of ligand solution (PGD_2, 10 nM, final concentration) was added to the lower chamber of a 96-well chemotaxis chamber (Diameter = 5 mm, #106-5, Neuro Probe). Cells were preincubated with various concentrations of ramatroban at 37 °C for 10 minutes. Chemotaxis is then allowed to occur in a humidified incubator at 37 °C, 5% CO_2 for 2 hours. The number of cells migrating into the lower chamber was

counted by FACScan (Becton-Dickinson). Ramatroban inhibited PGD_2-induced chemotaxis of human eosinophils in a dose-dependent manner with IC_{50}=50 nM.

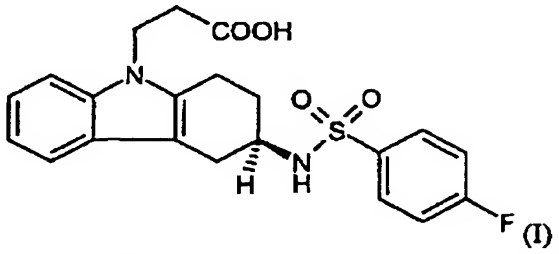
EXAMPLE 6

Human mononuclear cells were isolated from heparinized venous blood of healthy donors by laying the blood on Mono-Poly Resolving Medium (ICN Biomedicals, Co.Ltd) and centrifuging it at 400 x g for 30 min. at toom temperature. After centrifugation, $CD4^+$ T lymphocytes were purified from mononuclear cells by using $CD4^+$ T cell isolation kit (Miltenyi Biotec GmbH).

Human $CD4^+$ T lymphocytes were washed with PBS and resuspended in chemotaxis buffer (20 mM HEPES pH 7.6, 0.1% BSA, Hanks' solution) at 6 x 10^6 cells/ml. Fifty µl of the cell suspension (3 x 10^5 cells/well) was then dispensed into the upper chamber and 30 µl of ligand solution (PGD_2, 10 nM, final concentration) was added to the lower chamber of a 96-well chemotaxis chamber (Diameter = 3 mm, #106-3, Neuro Probe). Cells were preincubated with various concentrations of compound at 37 °C for 10 minutes. Chemotaxis is then allowed to occur in a humidified incubator at 37 °C, 5% CO_2 for 4 hours. The number of cells migrating into the lower chamber was counted by FACScan (Becton-Dickinson). Ramatroban inhibited PGD_2-induced chemotaxis of $CD4^+$ T lymphocytes in a dose-dependent manner with IC_{50}= 60 nM.

CLAIMS

(1) A CRTH2 modulator comprising the compound represented by the formula (I):



(I)

(+)-(3R)-3-(4-fluorobenzenesulfonamido)-1,2,3,4-tetrahydrocarbazole-9-propionic acid or a pharmaceutical acceptable salt thereof as an effective agent.

(2) A CRTH2 modulator as claimed in claim 1, wherein said CRTH2 modulator is effective for treating or preventing eosinophil-related diseases or basophil-related diseases.

(3) A CRTH2 modulator as claimed in claim 2, wherein said eosinophil-related diseases are one selected from the group consisting of Churg-Strauss syndrome and sinusitis.

(4) A CRTH2 modulator as claimed in claim 2, wherein said basophil-related diseases are one selected from the group consist of basophilic leukemia and basophilic leukocytosis.

(5) Use of the compound represented by the formula (I) as claimed in claim 1, for manufacturing a medicament for the treatment and/or prophylaxis of eosinophil-related diseases or basophil-related diseases.

(6) Process for controlling eosinophil-related diseases or basophil-related diseases in humans and animals by administration of an effective amount of the compound represented by the formula (I).

The Patent Office

INVESTOR IN PEOPLE

Application No:	GB 0211396.7 14	**Examiner:**	Lee Ellison
Claims searched:	1-6	**Date of search:**	11 June 2003

Patents Act 1977 : Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1 at least	Chemical Abstract Acc. No. 2000:353360 (CHUNG), see abstract.
X	1 at least	Chemical Abstract Acc. No. 2002:34819 (MOTOBAYASHI *et al*), see abstract.

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC[V]:

A5B

Worldwide search of patent documents classified in the following areas of the IPC[7]:

A61K; A61P

The following online and other databases have been used in the preparation of this search report:

CAS-ONLINE; EPODOC; WPI; PAJ